

Investigating the Antibacterial Properties of Inverse Vulcanized Sulfur Polymers

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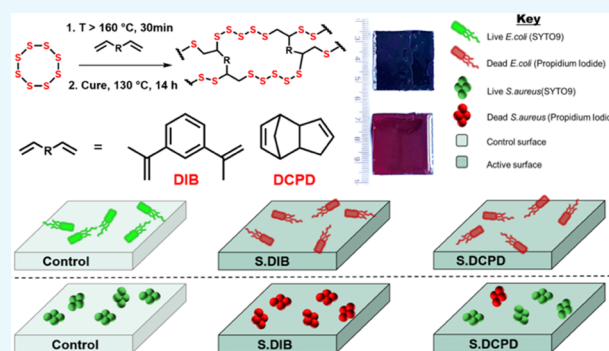


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Supporting Information

ABSTRACT: More than 60 million tons of sulfur are produced as a byproduct of the petrochemical industry annually. Recently, the inverse vulcanization process has transformed this excess sulfur into functional polymers by stabilization with organic cross-linkers. These interesting new polymers have many potential applications covering diverse areas. However, there has been very little focus on the potential of these high-sulfur polymers for their antibacterial properties. These properties are examined here by exposing two common bacteria species, *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*), to two structurally different, inverse vulcanized sulfur polymers: sulfur-*co*-diisopropenyl benzene (S-DIB) and sulfur dicyclopentadiene (S-DCPD). We report the highest bacteria log reduction (>log 4.3) of adhered bacterial cells (*S. aureus*) to an inverse vulcanized sulfur polymer to date and investigate the potential pathways in which antibacterial activity may occur.



INTRODUCTION

Synthetic polymers are ubiquitous and among the most extensively manufactured materials on earth. The environmental impact and sustainability of any alternative synthetic polymer are therefore important to consider. However, the majority of synthetic polymers are produced from limited resources derived from petrochemicals.¹ There is, therefore, a current goal in materials chemistry to identify sustainable building blocks that provide monomers generated from alternative sources, such as industrial waste.

Elemental sulfur is not only an abundant naturally occurring mineral; it is also produced as a byproduct from the petrochemicals industry. Sulfur is removed from crude oil and natural gas during refining.² Currently, the supply of sulfur outweighs demand, leading to vast unwanted stockpiles. In terms of materials chemistry, it would seem obvious to try to use this resource to make useful functional materials. Unfortunately, sulfur naturally occurs as S_8 , a small cyclic molecule that forms a brittle, crystalline powder, and thus not useful for making materials from. However, if sulfur is heated, it first melts and then polymerizes to form linear chains of sulfur atoms. The resultant ruby red polymer is a solid and could be used to make materials but it is not stable and readily depolymerizes back to S_8 rings at room temperature. A process termed inverse vulcanization has been shown to allow high-sulfur-content polymers to be stabilized against depolymerization by reacting the sulfur with organic small-molecule dienes, allowing sulfur contents up to 90 wt %. From this perspective,

there is current interest in exploiting this untapped, low-cost sulfur for materials.^{3–10}

In 2013, Pyun et al. first reported the inverse vulcanization process.⁴ In this process, molten sulfur acts as both a solvent and a monomer, eliminating the need for any solvents or initiators in traditional polymerization mechanisms. Since this study, many have shown that inverse vulcanized sulfur polymers can be prepared successfully with alternative cross-linkers.⁶ The low cost of sulfur and the unique properties exhibited by high-sulfur-content polymers, in comparison to those with a carbon backbone, have captured research interest,⁶ and inverse vulcanized sulfur polymers have received considerable attention for wide-ranging applications across Li–S batteries¹¹ heavy-metal capture,^{12,13} oil and water separation,¹⁴ lenses,¹⁵ thermal insulation,¹⁶ and self-healing polymers.¹⁷

One application that has received little attention is the antibacterial properties of high-sulfur-content polymers. Sulfur has been recognized throughout history as an antibacterial agent, given orally for bacterial infection and used topically as fungicides and bactericides for the treatment of cutaneous

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infections.¹⁸ Preventing biofilm formation is also of wider importance industrially, such as in coatings for ships' hulls and pipelines. Despite this, little experimental work has been carried out to investigate the antibacterial properties that high-sulfur-content polymers may possess.

More recently, Lienkamp et al. have studied the antibacterial surface properties of poly(sulfur-*co*-diisopropenyl benzene) (S-DIB).¹⁹ The findings show that polymer-covered surfaces kill up to 72% of *Escherichia coli* for a sample that contains 50 wt % sulfur.¹⁹ With this said, their findings suggest that S-DIB as a cross-linker is not ideal for antibacterial activity. Although promising, the Lienkamp study focuses on thin polymer film coatings and only one bacterial species, applied by spraying, and the antibacterial activity was assessed over a short time period (between 5 min and 4 h). With both *E. coli* and *Staphylococcus aureus* being serious causes of a variety of nosocomial infections,²⁰ the goal of the present study was to expose both *E. coli* and *S. aureus* to two different types of high-sulfur-content bulk polymer surfaces, thereby testing the effect on both Gram-positive and -negative species. To investigate the antibacterial activity, two polymers exhibiting different structural properties were chosen: S-DIB and sulfur dicyclopentadiene (S-DCPD) (Figure 1). S-DIB is a shape-

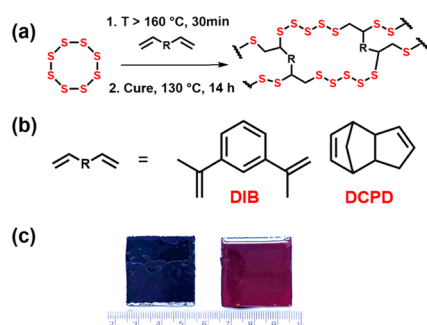


Figure 1. (a) Inverse vulcanization of elemental sulfur and cross-linker. (b) Cross-linkers used to prepare polymers. Left: 1,3-diisopropenyl benzene (DIB). Right: Dicyclopentadiene (DCPD) (c) Photograph showing the appearance of the polymer samples in bulk form. Left: Sulfur–DCPD copolymer (S-DCPD) Right: Sulfur–DIB copolymer (S-DIB), both prepared to dimensions of 30 x 30 x 3 mm³.

persistent, stable, hyperbranched polymer (Figure S1), whereas⁴ S-DCPD is a highly cross-linked polymer (Figure S2).¹² Here, we compare the antibacterial activity of two different types of inverse vulcanized sulfur polymers as robust, homogeneous, bulk solids rather than thin coatings that would be more subject to imperfections and damage. Inverse vulcanized S-DCPD and S-DIB polymers were successfully prepared at a ratio of 50 wt % sulfur content, similarly to previously published inverse vulcanization reactions (see Electronic Supporting Information (ESI) for details).^{4,12}

RESULTS AND DISCUSSION

Briefly, elemental sulfur was heated at 160 °C until it melted and became a pale orange liquid. Subsequently, the cross-linker (DCPD/DIB) was then added directly into molten sulfur and further heated for 20–30 min until a homogeneous mixture was formed. The mixture was then poured into a mold of dimensions of 30 × 30 × 3 mm³ and cured for 12–14 h at 130 °C to form solid squares suitable for further testing.

To determine if the resultant polymer surfaces possessed antibacterial effects, fluorescent microscopy using LIVE/DEAD BacLight was used to examine the response of *E. coli* to exposure to both polymer surfaces, S-DCPD and S-DIB surfaces, and to examine responses on polycarbonate (PC) control surfaces. *E. coli* was incubated on the three surfaces independently by immersing each surface in a 1:500 diluted nutrient broth (see ESI for further details). The cell density reached $\sim 1 \times 10^8$ cells/mL after incubation for 24 h. The cells were visualized on the various surfaces via fluorescent microscopy. There was no exponential growth of the *E. coli* and no biofilm formation was detected (see ESI for further details). Micrographs (Figures 2, S9–S17) indicated that S-

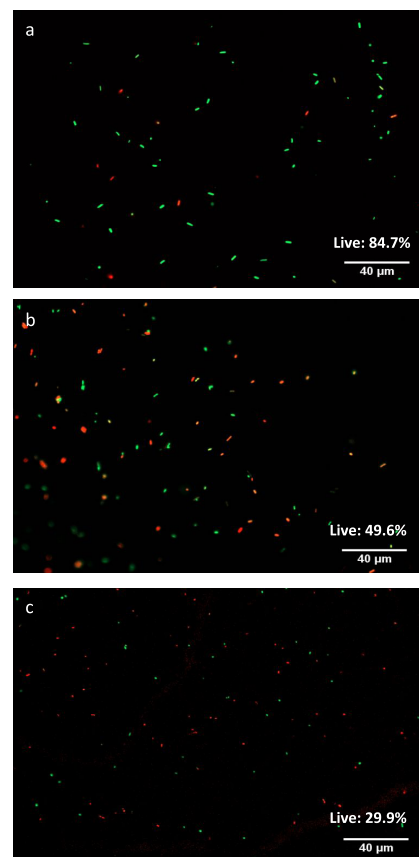


Figure 2. Fluorescent micrographs showing the attachment of *E. coli* (DSM 1576). Cells were stained with Syto 9 (green) and propidium iodide (red), detecting live and dead cells, respectively. Observation was achieved using a 100x objective lens. (a) polycarbonate control after 24 h incubation. (b) S-DCPD after 24 h incubation, (c) S-DIB after 24 h incubation. Scale bars represent 40 μm.

DIB surfaces significantly reduced the percentage of live cells (green) ($29.9 \pm 12.9\%$ survival) compared to both S-DCPD ($49.6 \pm 9.0\%$ survival) and the control sample ($84.7 \pm 4.1\%$). Furthermore, no noticeable variations were observed for the total cell numbers on the surface of the control and copolymer surfaces after 24 h.

To accurately quantify bacterial survival, we employed a standardized methodology (ISO 22196:2011 [see ESI for further details on modification]) for testing the antibacterial properties of a surface. Gram -ve (*E. coli* ATCC8739) and Gram +ve (*S. aureus* DSM347) bacterial strains were exposed

to both polymer surfaces and the number of viable cells recoverable from the surfaces was measured.

Briefly, surfaces were held in a Petri dish and seeded with 100 μ L of bacterial cell solution ($\sim 3 \times 10^6$ cells/mL) in a 1:100 (*S. aureus*) or 1:500 (*E. coli*) nutrient broth and covered with a polyethylene film. The inoculated surfaces were encased in a humidity chamber to limit surface evaporation and incubated at 37 $^{\circ}$ C for 24 or 48 h. To recover the bacteria, the surfaces were washed with 10 mL of soybean casein digest broth with lecithin and polyoxyethylene sorbitan monooleate. The recovered cells underwent serial dilutions and plating on plate count agar and were incubated for 48 h (see ESI for details).

The number of cells of both *E. coli* (>99.9% reduction, >3 log) and *S. aureus* (>99.9% reduction, >log 4.9) was significantly reduced by the S-DIB surface. (Figure 3, further

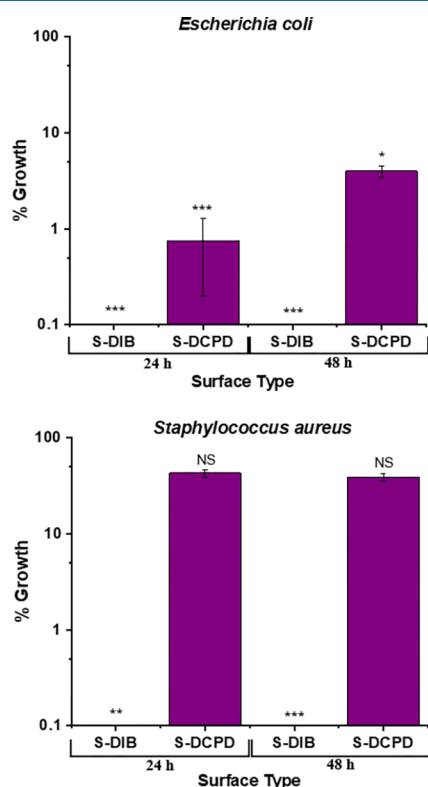


Figure 3. Antibacterial surface effect after 24 and 48 h, in comparison to the control, as measured by the growth of surface-adhered bacteria removed from the surface of the substrate using a neutralizing solution (SCDLP). Data have been normalized. Statistical analysis was carried on the log-transformed data. * (p -value < 0.05), ** (p -value < 0.01), *** (p -value < 0.001), NS (not significant). Error bars represent the standard deviation.

details in ESI). This is much higher in comparison to that in previous investigations, which report a 72% reduction for *E. coli* microorganisms on S-DIB (50 wt % sulfur).¹⁹ Both S-DIB and S-DCPD exhibit strong bactericidal effects on *E. coli* microorganisms in comparison to *S. aureus*, where only S-DIB exhibits a bactericidal effect on *S. aureus*. Scanning electron microscopy (SEM) imaging of the polymer surfaces, after exposure to the bacteria in equivalent conditions, does not show any significant biofilm formation (See ESI for details, Figures S18–S23), in agreement with the findings of confocal microscopy.

To further understand how the polymer surfaces may be having an antibacterial effect on the attached cells, the effect of sulfur leaching was assessed. Both bacterial strains were cultured in nutrient broth (NB) into which one of the three surface substrates was placed and these cultures were incubated at 37 $^{\circ}$ C (see ESI for details). From Figure 4, no

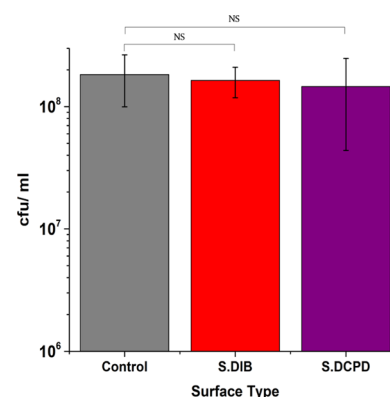


Figure 4. Assessment of antibacterial effects from substrate leaching based on bulk substrate submersion in broth culture. Cellular growth quantified by colony-forming units (cfu)/mL of broth culture after 24 h. Data have been normalized. NB Error bars represent the standard deviations from three independent replicates. NS, not significant; p -value, ≥ 0.05 .

significant difference in recovered c.f.u. was observed between the presence of the control ($1.8 \times 10^8 \pm 8.2 \times 10^7$, S-DIB ($1.6 \times 10^8 \pm 4.6 \times 10^7$), or S-DCPD ($1.4 \times 10^8 \pm 1.1 \times 10^8$)) substrates for *E. coli* cells in the planktonic phase. The difference in the cell viability between the samples is smaller than the standard deviations; this indicates that the release of any active sulfur-containing material into the liquid phase was negligible and did not affect cell viability. This is also supported by the live/dead staining (S9–S17), as a significant surface effect is observed for *E. coli* for both copolymers but there is no change in bacterial viability in the planktonic phase.

Parallel to this, both differential scanning calorimetry (DSC) and powder X-ray diffraction (PXRD) confirm that when both polymer surfaces are exposed to *E. coli* and *S. aureus*, no crystalline sulfur is formed and both polymers retain an amorphous character (Figures S3–S6). If the polymers were depolymerizing back to elemental sulfur (S_8), this would form crystals detectable by DSC and PXRD. Therefore, this suggests that the antibacterial effect arises from the action of the polymer itself, rather than the release of S_8 .

Why polysulfides exhibit antibacterial activity is not yet definitively understood; however, several mechanisms are suggested throughout the literature. On comparison of the antibacterial activity, S-DIB exhibits a greater reduction in cell numbers for both bacteria (Figure 3). Potential reasons for both polymers exhibiting different degrees of antibacterial activity could be due to the degree of cross-linking and the molar ratio of sulfur:cross-linker. It has been reported that polysulfides (RS_xR , $x \geq 3$) are toxic against bacteria, fungi, and particular types of human cells, although the reasons behind this are unknown.²¹ The central S–S bond in a polysulfide (RS_xR , $x \geq 4$) is weak in comparison to terminal S–S bonds, with bond dissociations of alkyltetrasulfides and disulfides being 146 and 293 kJ mol^{−1}, respectively.²¹ DCPD has a lower molecular mass than DIB (132 vs 162 g mol^{−1}) for the same

number of reactive double bonds. This means that for S-DIB, there are longer S–S chains (CS_xC , $x \geq 2$) between each carbon cross-link. The longer the polysulfide chains, the weaker the central S–S bond, and thus the more likely the occurrence of a homolytic S–S bond cleavage.^{21–23} This will lead to the formation of CS_x .²¹ The literature has shown this species to have great biological importance when killing bacteria, although specific reaction pathways are still unknown.^{21,23} Although little experimental data have been published with regard to the effect of sulfur-containing compounds against differentiated microorganisms, it is apparent that polysulfides appear to have similar effects against Gram-positive and -negative bacteria.^{24,25} However, the presence of low-concentration sulfides has been shown to provide some microorganisms, such as *S. aureus*, with protection against oxidative stress and certain antibacterial compounds.^{26,27} This could explain why we see subtle differences in *S. aureus* survival for S-DCPD treatment but would need to be further studied to confirm this scenario with the sulfur copolymers used here.

Other reported potential mechanisms for how polysulfides may kill bacteria are thiolation reactions, hydrophobic interactions, or S_x transfer reactions, which all involve leaching, and in some cases, the formation of crystalline sulfur.²¹ As the data in this study suggest that no significant amounts of material containing sulfur are leached and polymers retain an amorphous character, the most likely mechanism of action is homolytic bond cleavage.²¹ This high degree of stability of sulfur within the cross-linked bulk material is a desirable feature of long-term antibacterial surfaces. Surfaces associated with leaching or product release have a fixed lifetime based on the finite amount of antibacterial compounds present.²⁸ Furthermore, the creation of a potential concentration gradient or a local decrease in antibacterial compounds over time can lead to issues of antimicrobial resistance (AMR), and in vivo decreases in antibacterial drug concentrations are often associated with detrimental effects surrounding the host's immune response. However, a constitutively active antibacterial surface would be of more benefit both financially and for a reduction in hospital-acquired infections or industrial contamination, particularly with respect to AMR.

Bacterial infections have been considered to be one of the greatest threats to human health and are becoming more problematic due to increasing AMR.²⁸ With endotracheal tubes, vascular and urinary catheters, and hip prosthetics being responsible for more than half of the nosocomial infections in the United State,^{21,28} it is important to research different materials that may exhibit antibacterial properties for future development, particularly with the increasing prevalence of AMR. We have demonstrated the ability of inverse vulcanized sulfur polymers as bulk substrates to act as antibacterial surfaces against *E. coli* and *S. aureus*, the causes of common persistent bacterial infections. This study reports the highest log reduction ($>\log 4.3$) of both bacteria on different inverse vulcanized polymers to date. To fully translate this to a biological setting, complete knowledge of how the surfaces kill bacteria and the comparison of different high-sulfur-content polymers are essential. Here, we provide a vital investigation comparing two structurally different polymers and highlight key experiments that can help us identify the potential mechanisms of how these surfaces exhibit an antibacterial effect. It is interesting to note that while the mechanism of

action is not yet known with certainty, it is not inhibited by the thick peptidoglycan layer of Gram-positive bacteria.

■ CONCLUSIONS

In summary, we show the activity of two high-sulfur-content polymers, as bulk solids, against both Gram-negative and -positive bacteria. S-DIB is seen to have greater activity than S-DCPD; this could be attributed to S-DIB having a higher sulfur rank (the number of sulfur atoms in between each carbon cross-link, CS_x), therefore having a weaker central S–S bond promoting homolysis.

The low cost of availability of sulfur on a vast scale provides the potential for use as antibacterial materials and surfaces in bulk applications that would not be possible for more expensive or complex materials. The promising results found already and the difference in efficacy between these cross-linkers against two bacterial strains suggest that the broader antibacterial effect of sulfur polymers may be further improved in the future and certainly warrants further investigation and development. Future studies into the optimization and mechanism of the effect of these are needed, as well as an assessment of their safety within a hospital environment.

■ EXPERIMENTAL SECTION

Materials. 1,3-Diisopropenyl benzene (DIB) and dicyclopentadiene (DCPD) were purchased from Tokyo Chemicals Industry. Sulfur was purchased as 25 kg sacks from Brenntag. *E. coli* DMS 1576 and *S. aureus* DSM 346 strains provided by the University of Liverpool were used for the antimicrobial surface tests.

■ CHARACTERIZATION

X-Ray Diffraction (PXRD). Powder X-ray Diffraction (PXRD) patterns were carried out on samples using a PAN analytical X'pert powder diffractometer using Cu K α radiation.

Differential Scanning Calorimetry (DSC). Differential scanning calorimetry was carried out using Q2000 DSC (TA instruments). The method was a heat/cool/heat process for three cycles, heating to 150 °C and cooling to –80 °C at a heating rate of 5 °C/min with Tzero Hermetic pans.

Scanning Electron Microscopy (SEM). Scanning electron microscopy (SEM) was performed using a Hitachi S-4800 cold-field emission scanning electron microscope (FE-SEM). Samples were prepared by sticking them to the SEM stub using conductive silver adhesive paint. The sample was then coated with chrome using a current of 120 mA for 15 s to give approximately 15 nm chrome coatings using a Quorum S150T ES sputter coater. Imaging was conducted at a working distance of between 7.9 and 8.5 mm at an accelerating voltage of 1.5 kV.

■ METHODS

Synthesis of S-DIB and S-DCPD. Sulfur (10 g) was added to a 40 mL glass vial equipped with a magnetic stirrer bar and heated on a hot plate to 165 °C. Molten sulfur was formed (transparent, yellow solution) and, to this, 1,3-diisopropenyl benzene (DIB)/dicyclopentadiene (DCPD) (10 g) was added to the mixture via a pipette. The reaction mixture was heated at 165 °C until homogeneous (15–20 min). The product was then immediately transferred from the glass vial into a silicone mold of dimensions of 30 × 30 × 3 mm³. This was then cured at 130 °C for 14–16 h.

Bacteria Preparation. Bacterial strains were stored on nutrient agar containing 10 g L⁻¹ peptone, 5 g L⁻¹ NaCl, 2 g L⁻¹ yeast extract, 5 g L⁻¹ meat extract, and 15 g L⁻¹ agar at pH 7.1 ± 0.1. A scrape of bacteria was transferred to fresh nutrient agar and incubated for 37 °C for 18 h; this was then subsequently repeated. A loopful of agar-grown bacteria was transferred to a nutrient broth (1:500 dilution for *E. coli* and 1:100 dilution for *S. aureus*) containing 5 g L⁻¹ meat extract, 10 g L⁻¹ peptone (enzymatic digest of casein), 5 g L⁻¹ sodium chloride, and 15 g L⁻¹ agar at pH 7 ± 0.2. The bacterial cells were homogeneously suspended by vortexing for 10 s and water bath sonication for 10 s. 50 kHz (Grant Ultrasonic XB3). Bacterial enumeration was conducted using a calibration curve from the spectrophotometer value. The bacterial suspension was then adjusted to the desired optical density to achieve a target concentration of 3 × 10⁶ cells mL⁻¹.

Fluorescent Imaging. Testing was conducted on the control (polycarbonate), SDIB50:50, and SDCPD50:50 surfaces (30 × 30 mm²) and sterilized by submersion in 70% ethanol for 5 min; then, ABS ethanol 10 s. *E. coli* was grown overnight in LB Broth (15 mL), subcultured into fresh LB, and grown until an OD₆₀₀ of 0.4 was achieved. This subculture (10 mL) was centrifuged at 5000 g for 5 min. The supernatant was discarded and the pellet was resuspended in 30 mL of 1:500 diluted LB Broth at a concentration of approximately 10⁷ cells mL⁻¹. The surface was submerged in the cell suspension for 24 h. After incubation, the cell suspension was removed and the surface was gently washed with 0.85% sodium chloride (25 mL) three times. Live/Dead BacLight Bacterial Viability Kit L7007 was prepared by placing 1.5 μL of Syto 9 and 1.5 μL of propidium iodide in 1 mL of 0.85% sodium chloride. From this prepared stain, 1 mL was placed directly onto the surface and incubated in the dark for 15 min. The surface was washed with 25 mL of 0.85% sodium chloride and then imaged using a Zeiss Plan Apochromat 40x/1.0 DIC VIS-IR objective (Zeiss Axio Imager 2 microscope).

Assessment of Sulfur Leaching. Both bacterial strains were inoculated in nutrient broth (NB) at a concentration of 10⁵ cell/mL and a final volume of 30 mL. Substrates were added to the broth and incubated at 37 °C for 24 h. The growth was monitored by determining absorbance at 600 nm and an aliquot of 200 μL was serially diluted and plated on NA using the Miles and Misra method for the enumeration of colony-forming units (CFU). Data are shown in Figure 4 of the paper after normalization to set the control as 100%.

Surface Preparation and ISO Standard Testing. Testing was conducted on the control (polycarbonate), SDIB50:50, and SDCPD50:50 surfaces (30 × 30 mm²) and sterilized by submersion in 70% ethanol for 5 min and then ABS ethanol for 10 s. The surfaces were subsequently dried in a sterile environment using the aseptic technique. The test surface was placed in a Petri dish with an autoclaved sponge (20 × 20 mm²) containing 3 mL of sterilized H₂O. The bacterial suspension (100 μL, 3 × 10⁶ cell mL⁻¹) was then placed on the test surface and covered with a polyethylene film (20 × 20 mm²). The Petri dish was placed in a humidity chamber for 24 h. The surface was washed using a serological pipette uptaking and releasing soybean casein digest broth with lecithin and polyoxyethylene sorbitan monooleate (SCDLP broth, 10 mL (10×)). The resulting wash was then ten-fold serially diluted to 10⁻⁶ using sterile phosphate-buffered solution. The dilutions (1 mL) were added to 15 mL of plate count agar containing 2.5 g L⁻¹ yeast extract, 5 g tryptone

L⁻¹, 1 g glucose L⁻¹, and 15 g agar L⁻¹ at pH 7.1 ± 0.1 and incubated at (35 ± 1) °C for 40 to 48 h (any modifications to the ISO 22196 were stated in the methods section; however, the protocol was followed as closely as possible). Data are shown in Figure 3 of the main paper after normalization to set the control as 100%.

Bacteria Enumeration and Statistical Analysis. For each dilution series, the colony number was recorded and converted to recovered bacteria using the formula CFU/mL = (colony number × dilution factor) × 10. The antibacterial activity was calculated using the following formula

$$R = [\log(B/A) - \log(C/A)] = [\log(B/C)]$$

where *R* represents the antibacterial activity, *A* is the average number of viable bacteria immediately after inoculation on the control specimen, *B* is the average number of viable bacteria on the control specimen after 24 h, and *C* is the average number of viable bacteria on the antibacterial specimen after 24 h.

Fixation of Bacterial Cells for SEM Analysis. Surfaces were prepared as described in the "Surface preparation and ISO standard testing" section and then washed with PBS three times. The bacteria on the surface were then fixed with a 2.5% glutaraldehyde solution in sterile PBS for 4 h. The bacteria were then dehydrated in increasing concentrations of ethanol (30, 50, 75, 90, 95, and 100 v/v %) by soaking for 5 min in each ethanol concentration. The samples were dried in air and stored at 4 °C before SEM imaging.

Note on Lighting Conditions. All bacterial testing was carried out under ambient indoor lighting. While the light levels were relatively low, it should be noted that incident light, particularly UV light, might influence the frequency of S–S bond cleavage, and therefore bactericidal activity.

■ ASSOCIATED CONTENT

■ Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsomega.9b04267>.

Data of DSC, PXRD, cell studies, fluorescent micrographs, and SEM; reaction of sulfur with DIB, DCPD; PXRD pattern samples of S-DCPD, S-DIB; bacterial coverage of sulfur copolymer surfaces; fluorescent micrograph of *E. coli* (DSM 1576) (PDF)

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Notes

The authors declare no competing financial interest.

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REFERENCES

- (1) Zhu, Y.; Romain, C.; Williams, C. K. Sustainable Polymers from Renewable Resources. *Nature* **2016**, *540*, 354–362.
- (2) Kutney, G. *Sulfur: History, Technology, Applications & Industry*; ChemTec Pub: Toronto, 2007.
- (3) Boyd, D. A. Sulfur and Its Role In Modern Materials Science. *Angew. Chem., Int. Ed.* **2016**, *55*, 15486–15502.
- (4) Chung, W. J.; Griebel, J. J.; Kim, E. T.; Yoon, H.; Simmonds, A. G.; Ji, H. J.; Dirlam, P. T.; Glass, R. S.; Wie, J. J.; Nguyen, N. A.; Guralnick, B. W.; Park, J.; Somogyi, A.; Theato, P.; Mackay, M. M.; Sung, Y.; Char, K.; Pyun, J. The Use of Elemental Sulfur as an Alternative Feedstock for Polymeric Materials. *Nat. Chem.* **2013**, *5*, 518–524.
- (5) Griebel, J. J.; Glass, R. S.; Char, K.; Pyun, J. Progress in Polymer Science Polymerizations with Elemental Sulfur: A Novel Route to High Sulfur Content Polymers for Sustainability, Energy and Defense. *Prog. Polym. Sci.* **2016**, *58*, 90–125.
- (6) Worthington, M. J.; Kucera, R. L.; Chalker, J. M. Green Chemistry and Polymers Made from Sulfur. *Green Chem.* **2017**, *19*, 2748–2761.
- (7) Lim, J.; Pyun, J.; Char, K. Recent Approaches for the Direct Use of Elemental Sulfur in the Synthesis and Processing of Advanced Materials. *Angew. Chem., Int. Ed.* **2015**, *54*, 3249–3258.
- (8) Nguyen, T. B. Recent Advances in Organic Reactions Involving Elemental Sulfur. *Adv. Synth. Catal.* **2017**, *359*, 1066–1130.
- (9) Chalker, J. M.; Worthington, M. J.; Lundquist, N. A.; Esdaile, L. J. Synthesis and Applications of Polymers Made by Inverse Vulcanization. *Top. Curr. Chem.* **2019**, *377*, 1–27.
- (10) Zhang, Y.; Glass, R. S.; Char, K.; Pyun, J. Recent Advances in the Polymerization of Elemental Sulphur, Inverse Vulcanization and Methods to Obtain Functional Chalcogenide Hybrid Inorganic/Organic Polymers (CHIPs). *Polym. Chem.* **2019**, *10*, 4078–4105.
- (11) Zhang, Y.; Griebel, J. J.; Dirlam, P. T.; Nguyen, N. A.; Glass, R. S.; Mackay, M. E.; Char, K.; Pyun, J. Inverse Vulcanization of Elemental Sulfur and Styrene for Polymeric Cathodes in Li-S Batteries. *J. Polym. Sci., Polym. Chem.* **2017**, *55*, 107–116.
- (12) Parker, D. J.; Jones, H. A.; Petcher, S.; Cervini, L.; Griffin, J. M.; Akhtar, R.; Hasell, T. Low Cost and Renewable Sulfur-Polymers by Inverse Vulcanisation, and Their Potential for Mercury Capture. *J. Mater. Chem. A* **2017**, *5*, 11682–11692.
- (13) Worthington, M. J. H.; Kucera, R. L.; Albuquerque, I. S.; Gibson, C. T.; Sibley, A.; Slattery, A. D.; Campbell, J. A.; Alboaiji, S. F. K.; Muller, K. A.; Young, J.; Adamson, N.; Gascooke, J. R.; Jampiah, D.; Sabri, Y. M.; Bhargava, S. K.; Ippolito, S. J.; Lewis, D. A.; Quinton, J. S.; Ellis, A. V.; Johs, A.; Bernardes, G. J. L.; Chalker, J. M. Laying Waste to Mercury: Inexpensive Sorbents Made from Sulfur and Recycled Cooking Oils. *Chem. Eur. J.* **2017**, *23*, 16219–16230.
- (14) Worthington, M. J.; Shearer, C. J.; Esdaile, L. J.; Campbell, J. A.; Gibson, C. T.; Legg, S. K.; Yin, Y.; Lundquist, N. A.; Gascooke, J. R.; Albuquerque, I. S.; Shapter, J. G.; Andersson, G. G.; Lewis, D. A.; Bernardes, G. J. L.; Chalker, J. M. Sustainable Polysulfides for Oil Spill Remediation: Repurposing Industrial Waste for Environmental Benefit. *Adv. Sustainable Syst.* **2018**, *2*, No. 1800024.
- (15) Griebel, J. J.; Nguyen, N. A.; Namnabat, S.; Anderson, L. E.; Glass, R. S.; Norwood, R. A.; Mackay, M. E.; Char, K.; Pyun, J. Dynamic Covalent Polymers via Inverse Vulcanization of Elemental Sulfur for Healable Infrared Optical Materials. *ACS Macro Lett.* **2015**, *4*, 862–866.
- (16) Abraham, A. M.; Kumar, S. V.; Saeed, A. M. Porous Sulphur Copolymer for Gas-phase Mercury Removal and Thermal Insulation. *Chem. Eng. J.* **2018**, *332*, 1–7.
- (17) Parker, D. J.; Chong, S. T.; Hasell, T. Sustainable Inverse-Vulcanised Sulfur Polymers. *RSC Adv.* **2018**, *8*, 27892–27899.
- (18) Saida, K.; Nomoto, Y.; Okauchi, H.; Ogiwara, H.; Nishimoto, K. Influences of Phosphorus and Sulphur on Ductility Dip Cracking Susceptibility in Multipass Weld Metal of Alloy 690. *Sci. Technol. Weld. Joining* **2012**, *17*, 1–8.
- (19) Deng, Z.; Hoefling, A.; Théato, P.; Lienkamp, K. Surface Properties and Antimicrobial Activity of Poly (Sulfur- Co -1, 3-Diisopropenylbenzene) Copolymers. *Macromol. Chem. Phys.* **2018**, *219*, No. 1700497.
- (20) Ejrnæs, K. Bacterial Characteristics of Importance for Recurrent Urinary Tract Infections Caused by *Escherichia Coli*. *Dan. Med. Bull.* **2011**, *58*, No. B4187.
- (21) Münchberg, U.; Anwar, A.; Mecklenburg, S.; Jacob, C. Polysulfides as Biologically Active Ingredients of Garlic. *Org. Biomol. Chem.* **2007**, *5*, 1505–1518.
- (22) Pickering, T. L.; Saunders, K. J.; Tobolsky, A. V. Disproportionation of Organic Polysulfides. *J. Am. Chem. Soc.* **1967**, *89*, 2364–2367.
- (23) Yin, M. C.; Chang, H. C.; Tsao, S. M. Inhibitory Effects of Aqueous Garlic Extract, Garlic Oil and Four Diallyl Sulphides against Four Enteric Pathogens. *J. Food Drug Anal.* **2002**, *10*, 120–126.
- (24) Lim, H.; Kubota, K.; Kobayashi, A.; Sugawara, F. Sulfur-Containing Compounds from *Scorodocarpus Borneensis* and Their Antimicrobial Activity. *Phytochemistry* **1998**, *48*, 787–790.
- (25) Xu, Z.; Qiu, Z.; Liu, Q.; Huang, Y.; Li, D.; Shen, X.; Fan, K.; Xi, J.; Gu, Y.; Tang, Y.; Jing, J.; Xu, J.; He, J.; Gao, X.; Liu, Y.; Koo, H.; Yan, X.; Gao, L. Converting Organosulfur Compounds to Inorganic Polysulfides against Resistant Bacterial Infections. *Nat. Commun.* **2018**, *9*, No. 3713.
- (26) Weikum, J.; Ritzmann, N.; Jelden, N.; Klöckner, A.; Herkersdorf, S.; Josten, M.; Sahl, H.-G.; Grein, F. Sulfide Protects *Staphylococcus Aureus* from Aminoglycoside Antibiotics but Cannot Be Regarded as a General Defense Mechanism against Antibiotics. *Antimicrob. Agents Chemother.* **2018**, *62*, e00602–e00618.
- (27) Shatalin, K.; Shatalina, E.; Mironov, A.; Nudler, E. H₂S: A Universal Defense Against Antibiotics in Bacteria. *Science* **2011**, *334*, 986–990.
- (28) Murata, H.; Koepsel, R. R.; Matyjaszewski, K.; Russell, A. J. Permanent, Non-Leaching Antibacterial Surfaces — 2: How High Density Cationic Surfaces Kill Bacterial Cells. *Biomaterials* **2007**, *28*, 4870–4879.